

Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014

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Human enterovirus D68 (EV-D68) is known to be associated with mild to severe respiratory infections. Recent reports in the United States and Canada of acute flaccid paralysis (AFP) in children with detection of EV-D68 in respiratory samples have raised concerns about the aetiological role of this EV type in severe neurological disease. This case study is the first report of AFP following EV-D68 infection in Europe.

We report the first case of acute flaccid paralysis (AFP) following enterovirus-D68 (EV-D68) infection in Europe. The United States (US) and Canada are currently experiencing nationwide outbreaks of EV-D68 infections associated with severe respiratory diseases especially in children with underlying respiratory disease that began in mid-August 2014 [1,2]. Concomitantly, clusters of neurological illness characterised by AFP with anterior myelitis have been reported in the US and Canada [3,4]. The detection of EV-D68 in nasopharyngeal specimens of some affected children raises the question of a possible link between EV-D68 infections and severe neurological disease.

Human enterovirus D68 belongs to the enterovirus D species within the *Enterovirus* genus in the *Picornaviridae* family. Biologically close to rhinoviruses, EV-D68 has been mainly associated with acute respiratory infection with clinical presentation ranging from mild to severe disease requiring intensive care [5–11].

Case report

The patient was a previously healthy four year-old boy who was initially taken to his general practitioner's surgery for headache and vomiting on 20 September 2014. On 22 September 2014, he presented with a febrile meningeal syndrome without any sign of encephalitis.

Cerebrospinal fluid (CSF) showed pleocytosis (190 leucocytes: 92% of lymphocytes, norm <10 leucocytes)

FIGURE 1

Spinal magnetic resonance image, acute flaccid paralysis case following enterovirus D68 infection, France, 2014

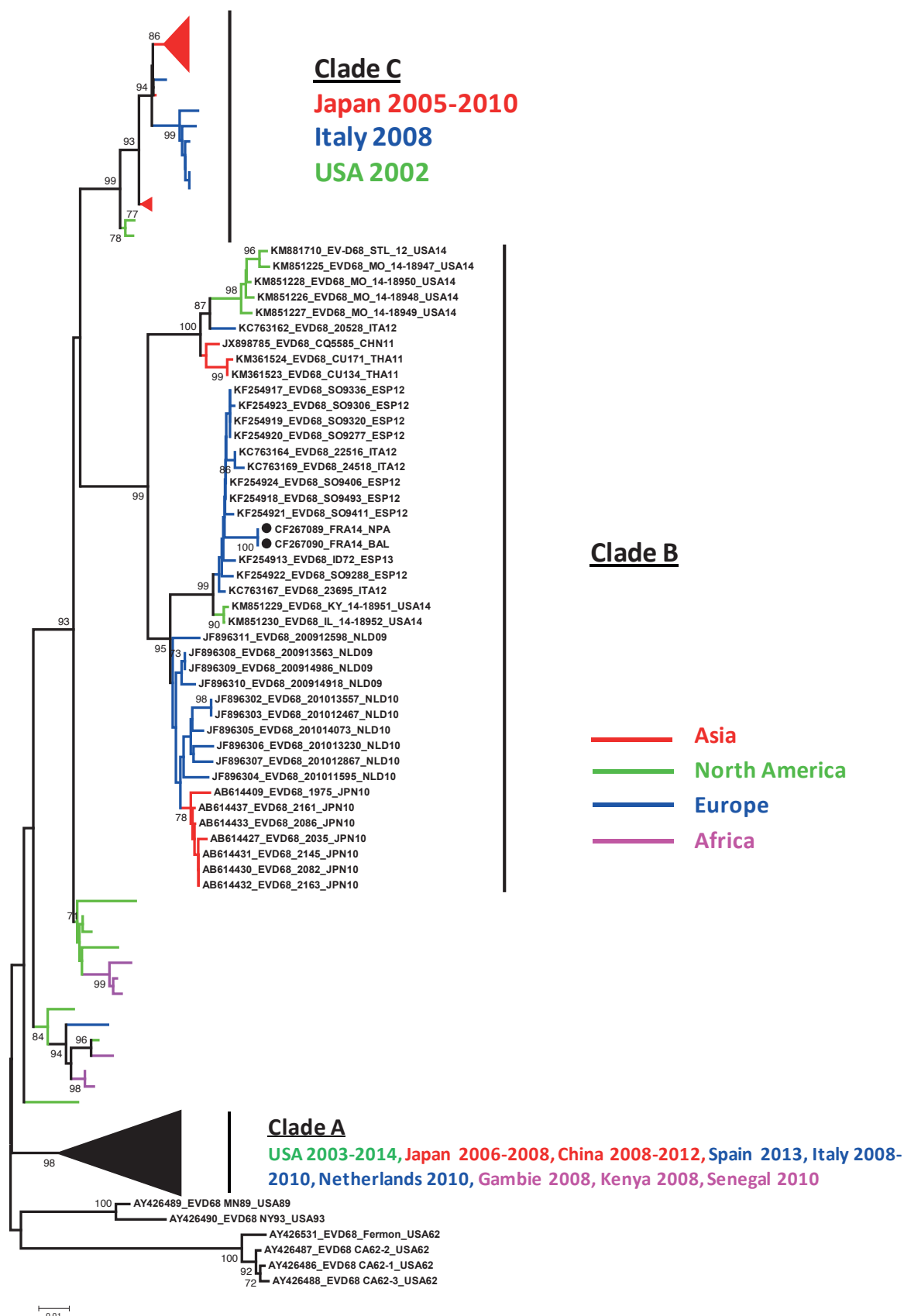


Gadolinium enhancement of the ventral nerve roots of the cauda equina is shown (arrows).

with normal protein and glucose levels, consistent with aseptic meningitis. On 26 September, he was transferred to the paediatric Intensive Care Unit for mechanical ventilation and fluid restoration because of acute respiratory distress and haemodynamic failure. Chest X-ray and thoracic tomodensitometry (TDM) confirmed bilateral pneumonia. Intravenous antibiotic therapy (ceftriaxone) was initiated. Acute myocarditis with apical hypokinesia was assessed by ultrasound examination. Levels of N-terminal of the prohormone brain natriuretic peptide (NT proBNP) and troponine Ic were up to 2,925 ng/L (norm: <450 ng/L) and 3.06 µg/L (norm: <0.045 µg/L), respectively. Leucocytes were elevated two–three times above the upper limit of normal (20,630/mm³, norm: 4,500–13,000/mm³) with 16,880/mm³ of polynuclear cells (norm: 1,500–8,000/mm³). C-reactive protein level was elevated (75.8 mg/l, norm: <10 mg/l). The patient received 0.5 g/kg/day of

FIGURE 2

Phylogeny of enterovirus D68 sequences inferred with 190 VP1 sequences



The phylogenetic tree was constructed by the neighbor-joining method and evaluated with 1,000 bootstrap pseudoreplicates, using MEGA5. Only bootstrap values > 70 % are indicated. Genetic distances were calculated with Tamura-Nei's model of evolution and branch length is drawn to the indicated scale (proportion of nucleotide substitutions per site). Sequences were 740 pb long and started to nucleotide 1 relative to the VP1 gene of the Fermon prototype strain. For clarity, taxon names are not fully included in the tree except for clade B. The strains identified in the nasopharyngeal aspirate (CF267089_FRA14_NPA) and in the bronchoalveolar fluid (CF267089_FRA14_BAL) are labeled with a filled circle. Geographical origins and time of isolation of strains are indicated by the ISO-code abbreviation followed by the year of isolation.

intravenous immunoglobulin (IVIG) and milrinone for four days. On 27 September, he presented with flaccid tetraparesis and dysphagia. Cerebral magnetic resonance imaging (MRI) was normal but spinal MRI showed gadolinium enhancement of the ventral nerve roots of the cauda equina (Figure 1).

Somatosensory evoked potentials confirmed that only the motor pathway was affected. Acute polyradiculoneuritis was excluded because there was no albumino-cytological dissociation and no antiganglioside antibodies in the CSF. There was no paraneoplastic syndrome (whole body-TDM and biological tumour markers were negative) and no inflammatory disease. Plasmapheresis and IVIG were implemented to shorten the recovery period. As of 6 November, the child has only recovered partial mobility of the extremities and of his left arm.

The child had up-to-date immunisation against poliomyelitis. He had neither underlying respiratory illness nor previous history of chronic disease, immunodeficiency or tick exposure. He had not travelled recently outside France and had had no contact with anyone arriving from North America. No family member presented with respiratory symptoms.

Blood, pre-IVIG serum, urine, respiratory and stool samples were screened. Bacteriological investigations including cultures, serology and genome detection in blood and CSF yielded negative results (Table).

Virological screening consisting in viral genome detection for numerous neurotropic viruses including EVs of three consecutive CSF was negative, as were all serological tests (Table). Viral cultures were negative. Rhinovirus-EV genome was detected in nasopharyngeal aspirates, bronchoalveolar fluid (BAL) and a stool sample using a one-step RT-PCR with previously described primers targeting the 1A and 1B regions encoding the VP4-VP2 capsid proteins [12]. To distinguish between rhinoviruses and EVs, amplified products were subjected to direct sequencing as previously described [13]. Blast analysis confirmed by phylogenetic analysis with VP4-VP2 sequences of rhinovirus and EV prototype strains assigned the strains to EV-D68. Partial 1D gene encoding the VP1 capsid protein was amplified by semi-nested RT-PCR using EV-D68 specific primers described by Tokarz et al. [14] in respiratory and stool samples, and subsequently sequenced (accession number LN626610). Phylogenetic investigation with all available sequences of EV-D68 (as of 9 October 2014) indicated that the strains belonged to clade B, according to the classification previously described [14]. The VP1 sequences were genetically close to sequences of some of the EV-D68 strains detected in 2014 in the United States (Figure 2).

Discussion

While EV-D68 has to date been almost exclusively associated with respiratory diseases, investigations are currently underway to determine its role in the acute neurological illnesses that have been reported in children in the US [3] and in Canada [4] since August 2014. Nine EV-D68-associated deaths are currently being investigated at the US Centers for Disease Control and Prevention (CDC) to confirm or refute EV-D68 as the cause of death [15]; as of 5 November, no information has been released about the death's preceding symptoms. The case reported here meets the definition given by CDC to identify similar neurological manifestations characterised by acute onset of focal limb weakness occurring on or after 1 August 2014 and MRI showing a spinal cord lesion largely restricted to grey matter [16]. Common features with the cases reported in the US include (i) respiratory illness preceding development of neurological symptoms, (ii) a local epidemiological context of EV-D68 detection among children admitted to hospital for respiratory infections leading to asthma crisis (data not shown) and (iii) EV-D68 detection in respiratory samples. By contrast, to our knowledge, neither meningeal syndrome nor myocarditis and acute respiratory distress syndrome had been reported in the days preceding the onset of paralysis in the US patients. The enterovirus genome was not detected in the CSF of this patient and we cannot assert that EV-D68 was associated with meningitis. There are two case reports in the literature of EV-D68 infection associated with severe neurological disease as evidenced by detection in the CSF [17,18]. As in recent reports, the significance of EV-D68 association with AFP is hampered by the fact that it was only detected in respiratory or stool samples, in which enteroviruses can be detected many weeks after infection. However, the absence of detection in CSF does not necessarily rule out this possibility since poliovirus and EV-A71, two recognised neurotropic EVs, are not frequently recovered [19]. Further physiopathological studies may be needed to assess the neurotropism of EV-D68.

There are increasingly numerous reports of polio-like illnesses in the US (64 cases as of 30 October 2014) [15]. Surveillance of AFP cases has already been implemented as a measure in the global initiative to eradicate poliomyelitis and should allow rapid identification of similar neurological manifestations in association with EV-D68 infection [20]. However, determination of AFP aetiologies can be challenging, because of the absence of pathogen detection in the CSF. Investigation of AFP cases should include both EV screening of two stool samples collected ≥ 24 hours apart and < 14 days after symptom onset [21] and early and quick testing of diverse samples, especially upper respiratory samples, for infectious agents including EVs, to increase the chance to identify a pathogen. In the case of EV-D68 infections, the detection capabilities of the EV-D68 genome of commercial and in-house molecular methods should be assessed.

TABLE

Bacteriological and virological investigations performed on clinical specimens collected in the first week of the course of the illness, acute flaccid paralysis case following enterovirus D68 infection, France, 2014

Collection date (2014)	Samples	Microbiological investigations	Methods	Results
22 September	CSF	Bacterial culture		Negative
		HSV1-2, EV, Rhinovirus-EV, VZV, ADV, CMV, EBV, HPeV	Real-time (RT)-PCR ^a and classic RT-PCR ^b	Negative
	Throat swab	EV	Real-time RT-PCR and EV-68 specific semi-nested RT-PCR	Negative
	Stool	EV	Real-time RT-PCR and EV-68 specific semi-nested RT-PCR	EV-D68 positive
24 September	Serum	<i>Borrelia</i> , <i>Mycoplasma pneumoniae</i> , <i>Chlamydia pneumoniae</i>	Serological tests	Negative
		EV, HSV1-2 / Rickettsia	Real-time (RT)-PCR and EV-68 specific semi-nested RT-PCR	Negative
		Human immunodeficiency virus (HIV), Parvovirus B19, HSV1-2 / Rickettsia	Serological tests	Negative
	Rectal swabs	EV, Rhinovirus-EV, HPeV	Real-time (RT)-PCR and EV-68 specific semi-nested RT-PCR	Negative
		Rotavirus, ADV	Immuno-chromatography	Negative
	BAL	Influenzae virus A-B, RSV, hMPV, EV, Rhinovirus-EV, ADV, BoV, hPIV1-4, hCoV, HSV1-2, CMV	Real-time (RT)-PCR and EV-68 specific semi-nested RT-PCR	EV-D68 positive
		Bacterial culture		Negative
	Nasopharyngeal aspirate	Influenzae virus A-B, RSV, hMPV, EV, Rhinovirus-EV, ADV, BoV, hPIV1-4, hCoV	Real-time (RT)-PCR and EV-68 specific semi-nested RT-PCR	EV-D68 positive
		<i>Chlamydia pneumoniae</i> , <i>Mycoplasma pneumoniae</i>	Real-time PCR	Negative
	Urine	Bacterial culture		Negative
25 September	Whole blood	EV, HHV6, CMV, EBV, ADV, VZV	Real-time (RT)-PCR	Negative
	Serum	Parvovirus B19	Real-time PCR	Negative
		HAV, HBV, HCV, Measles, Mumps, Rubella	Serological tests	Negative
		Rickettsia	Real time PCR	Negative
26 September	CSF	EV, Rhinovirus-EV, HSV1-2, VZV, ADV, CMV, EBV, HHV6	Real-time (RT)-PCR and EV-68 specific semi-nested RT-PCR	Negative
		<i>Leptospira</i> sp., <i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Mycoplasma</i> sp., <i>Streptococcus agalactiae</i> , <i>Ureaplasma urealyticum</i> , <i>Bartonella</i> , <i>Borrelia</i> , <i>Rickettsia</i> , <i>Tropheryma Whipplei</i>	Real-time PCR	Negative

ADV: adenovirus; BAL: bronchoalveolar lavage; BoV: Bocavirus; CMV: cytomegalovirus; CSF: cerebrospinal fluid; EV: enterovirus; EBV: Epstein-Barr virus; HAV: hepatitis A virus; HBV: hepatitis B virus; hCoV: human coronavirus; HCV: hepatitis C virus; HHV: human herpes virus; hMPV: human metapneumovirus; HPeV: human parechovirus; hPIV: human parainfluenzae virus; HSV: herpes simplex virus; RSV: respiratory syncytial virus; VZV: varicella zoster virus.

^a For enteroviruses, a commercial pan-EV RT-PCR was used.

^b Classic RT-PCR was used for rhinovirus-EV genome detection [12].

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Authors' contributions

Mathieu Lang, Nadia Savy, Sarah Maridet and André Labbé were involved in the clinical management of the patient. The virological investigations were performed by Audrey Mirand, Cécile Henquell and Hélène Peigue-Lafeuille. Magnetic resonance imagery was interpreted by Renan Pérignon. Audrey Mirand wrote the first draft of the paper. All authors reviewed the manuscript critically.

Conflict of interest

None declared.

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